UNIVERSITI SAINS MALAYSIA

Second Semester Examination
Academic Session 2005/2006

April/Mei 2006

BTT 202E/3 – Techniques in Biotechnology
[Teknik-Teknik Bioteknologi]

Duration: 3 hours
Masa : [3 jam]

Please ensure that this examination paper contains EIGHT printed pages before you begin the examination.

Answer FIVE out of SIX questions, in English or Bahasa Malaysia.

Each question carries 20 marks.

Sila pastikan bahawa kertas peperiksaan ini mengandungi LAPAN muka surat yang bercetak sebelum anda memulakan peperiksaan ini.

Jawab LIMA daripada ENAM soalan yang diberikan dalam Bahasa Inggeris atau Bahasa Malaysia.

Tiap-tiap soalan bernilai 20 markah.

...2/-

Answer the following questions based on Figure 1.

1. [a] Design a pair of primers to amplify the non-structural protein 3 (NS3) gene. Specify the forward and the reverse primers and the bases where they are complemented. You are also required to introduce an *NdeI* (CATATG) site at the beginning of the gene and a *HindIII* (AAGCTT) site at the end of the gene. Make sure you have all the necessary elements so that the cloned gene could be expressed.

(5 marks)

[b] What kind of gene amplification method must you use? With the help of a diagram, explain the principles of your chosen method. (10 marks)

(10 marks)

[c] What would be the expected size of the amplified fragment?

(1 marks)

[d] What would happen if dideoxynucleotides (ddNTPs) were added into the amplification reaction mixture? Explain your answer.

(4 marks)

Jawab soalan-soalan berikut berdasarkan Rajah 1.

1. [a] *Rekabentukkan sepasang pencetus untuk mengamplifikasi gen protein bukan berstruktur (NS3). Tentukan pencetus ke hadapan dan pencetus ke belakang dan juga bes di mana pencetus-pencetus tersebut berkomplemen. Anda juga dikehendaki mewujudkan tapak pembatasan NdeI (CATATG) pada permulaan gen dan tapak pembatasan HindIII (AAGCTT) pada penghujung gen. Pastikan anda mempunyai unsur yang diperlukan untuk gen yang diklonkan dapat diekspreskan.*

(5 markah)

[b] *Apakah cara pengamplifikasi gen yang mesti anda guna? Dengan bantuan gambarajah, terangkan prinsip cara pengamplifikasi gen yang anda pilih.*

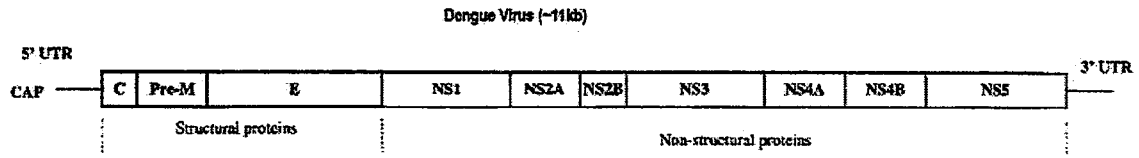
(10 markah)

[c] *Apakah saiz fragmen yang dijangkakan terhasil?*

(1 markah)

[d] *Apakah yang akan berlaku sekiranya 'dideoksinukleotidea (ddNTPs)' dimasukkan ke dalam campuran tindakbalas amplifikasi? Terangkan jawapan anda.*

(4 markah)

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LOCUS       DEN2CGA                 10723 bp ss-RNA     linear
DEFINITION  Dengue virus type 2 complete genome.
ACCESSION   M29095 M19727
COMMENT     Original source text: Dengue virus type 2
            cDNA to viral RNA.
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     CDS             97..10272
                     /note="viral polyprotein"
                     ----------
                     4522..6375
                     /product="nonstructural protein 3"
                     ----------
 4441 ctggtgatct caggactttt tcctgtatca ataccaatca cggcagcagc atggtacctg
 4501 tgggaagtga agaaacaacg ggctggagta ttgtgggatg tcccttcacc cccacccgtg
 4561 ggaaaggctg aactggaaga tggagcctat agaatcaagc aaaaagggat tcttggatat
 4621 tcccagatcg gagccggagt ttacaaagaa ggaacattcc atacaatgtg gcatgtcaca
 4681 cgcggcgctg ttctaatgca taaaggaaag aggattgaac catcatgggc ggacgttaag
 4741 aaagacctaa tatcatatgg aggaggctgg aagctagaag gagaatggaa ggaaggagaa
 4801 gaagtccagg tcttggcatt ggagcctgga aaaaatccaa gagccgtcca aacaaaacct
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 4921 acctcaggat cgccaatcat cgacaaaaaa ggaaaagttg tgggtcttta tggtaatggt
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 6421 ttcatgactc agaaggcaag agacgcactg gacaacttag cagtgctgca cacggctgaa
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Figure 1. Genomic organization of dengue virus. The sequence information was extracted from the NCBI database.

Rajah 1. Struktur genom virus denggi. Maklumat jujukan telah diambil dari pengkalan data NCBI.

2. [a] With the help of a diagram, explain the principles of agarose gel electrophoresis, including the staining step.

(8 marks)

[b] A student ran a plasmid sample in an agarose gel electrophoresis and found three bands. How was this possible?

(7 marks)

[c] A student performed restriction enzyme digestion on a plasmid and found no sign of digestion after running an agarose gel electrophoresis. Describe all possible explanations to this situation.

(5 marks)

2. *[a] Dengan bantuan gambarajah, terangkan prinsip elektroforesis gel agarosa, termasuk langkah pewarnaan.*

(8 markah)

[b] Seorang pelajar telah melarikan sampel plasmid pada elektroforisis gel agarosa dan mendapati tiga jalur telah terbentuk pada gel tersebut. Bagaimanakah ini boleh terjadi?

(7 markah)

[c] Seorang pelajar telah menjalankan pencernaan enzim pembatasan ke atas satu plasmid dan mendapati tiada pemotongan berlaku setelah dilakukan elektroforesis gel agarosa. Terangkan segala kemungkinan kepada keadaan ini.

(5 markah)

3. Describe the functions and the applications of the following enzymes:

[i] T4 DNA ligase
[ii] T4 polynucleotide kinase
[iii] Ribonuclease
[iv] Terminal deoxynucleotidyl transferase
[v] Dnase
[vi] Mung bean nuclease
[vii] DNA methylase
[viii] Reverse transcriptase
[ix] Alkaline phosphatase
[x] T4 DNA polymerase

(20 marks)

3. *Terangkan fungsi dan kegunaan enzim-enzim berikut:*

[i] T4 DNA ligase
[ii] T4 polynucleotide kinase
[iii] Ribonuclease
[iv] Terminal deoxynucleotidyl transferase
[v] Dnase
[vi] Mung bean nuclease
[vii] DNA methylase
[viii] Reverse transcriptase
[ix] Alkaline phosphatase
[x] T4 DNA polymerase

(20 markah)

4. [a] Write notes on techniques used to break bacterial cells.

(10 marks)

[b] Explain how diafiltration is carried out using ultrafiltration technology.

(5 marks)

[c] Explain the advantages and disadvantages of shake-flask and fermenter as bioreactors.

(5 marks)

4. [a] Tulis nota berkenaan teknik-teknik pemecahan sel bakteria.

(10 markah)

[b] Terangkan bagaimana "diafiltration" dilakukan menggunakan teknologi penurasan-ultra.

(5 markah)

[c] Terangkan kebaikan dan keburukan kelalang-goncang dan fermenter sebagai bioreaktor.

(5 markah)

5. Draw the flow-chart of the purification of a bacterial intracellular protease. Discuss each unit-process involved.

(20 marks)

5. Lakarkan carta-alir proses penulenan enzim protease intrasel bakteria. Bincangkan setiap proses unit yang terlibat.

(20 markah)

...8/-